Restriction Digestion of Lambda DNA

Aim: To investigate the efficiency and outcome of cutting single-digested lambda-DNA with the restriction enzyme *Eco*RI, using Wealtecs CB-1 Block Cooler as incubation system. *Eco*RI is a Type II restriction enzyme, isolated from E *coli*, that recognises and cuts the sequence:

G|AATT C
C TTAA|G

INSTRUCTION

Classification: Restriction enzymes, or restriction endonucleases, are proteins that recognize and cleave specific sequences of double stranded DNA (Mani *et al.*, 2005a). Most, but not all of the 3000 discovered restriction enzymes found so far, come from bacteria, where they serve as a protection system, defending bacteria from foreign DNA. Restriction enzymes are divided into three major groups based on different preferences associated with recognition/cutting-sites and environmental requirements, e.g. certain temperature, ionic strength and pH. *Type I* and *III restriction enzymes* recognise certain DNA sequences and cut some distance away, some times as far as 10 000 base pairs away, whereas *Type II restriction enzymes* cut sequences located at a close proximity to the recognition site. Most restriction enzymes recognise sequences that are relatively short, often 4-8 base pairs in length. Furthermore, Type II enzymes, which comprise the most abundant group of enzymes, often recognise palindrome sequences and cut within or adjacent to these sequences.

Applications: The discovery of restriction enzymes in the late 1960s has had an enormous impact on molecular biology research. Cutting large DNA-molecules with restriction enzymes makes it possible to purify homogenous DNA-fragments of defined lengths that can be subsequently enzymatically manipulated and analysed (Mani *et al.*, 2005a). Cut DNA-fragments can easily be ligated into DNA-molecules with corresponding ends, thereby making recombinant DNA-molecules. Due to the exact specificity of restriction enzymes, and the specific cleavage patterns generated when cut DNA is run on gels, restriction enzymes also enables for mapping of DNA. A common application of this is *restriction fragment length polymorphism* (RFLP), used for e.g. paternity testing. In RFLP, specific human genomic DNA-areas are cut by several restriction enzymes, and the fragments are subjected to electrophoresis. The generated fragment pattern is unique for

a given individual, but shares certain similarities with patterns generated by related individuals. Moreover, restriction enzymes can also be used to detect specific variations/mutations in DNA caused by a single nucleotide change, so called single nucleotide polymorphisms (SNPs) (Dear 2005). The SNP may generate a new cutting site, or it may result in the loss of a cutting site. Therefore, by cutting DNA with restriction enzymes directed at the site of a possible mutation and thereafter subjecting the fragments to electrophoresis, the gain or loss of a band tells whether a SNP is present or not.

Working conditions: Finding the optimal operating condition for restriction enzymes can be a hassle. *pH and salt-concentration* is very important, which is why companies selling restriction enzymes frequently also provide the appropriate buffer compatible with the enzyme. Some enzymes are very sensitive to certain ions, such as potassium or sodium, whereas others work in a wide range of ionic strengths. The divalent cat ion Mg^{2+} is required by most restriction enzymes. The correct *working temperature* is also important, simply because the bacteria that originally house the restriction enzyme have different temperature ranges within which they operate optimally. Many bacteria have operation optima around body temperature (37°C), but some prefer extreme heat or cool temperatures.

Figure 1: *Wealtec's CB-1 Block Cooler. With a temperature range from 0 to 70°, CB-1 covers a wide range of incubation applications within fields of molecular biology. CB-1 has 9 pre-set temperatures and customised temperature setting is extremely easy. An insulated lid and exact temperature control makes CB-1 perfect for e.g. restriction digestion applications.*

MATERIALS AND METHODS

A restriction mixture consisting of lambda DNA, completely digested by HindIII (Wealtec, Taipei, Taiwan), *Eco*RI restriction enzyme (10 U/µl; Fermentas, Glen Blunie, MD, U.S.A.), 10 X *Eco*RI digestion buffer (Fermentas), and nuclease free water (New England Biolabs, Ipswich, MA, U.S.A) was added to eppendorf tubes to a final volume of 20 µl for incubation at 37°C (pre-setting) for one hour in the block cooler (Wealtec).
In order to guarantee the correct incubation temperature, the block cooler had been pre-warmed for 30 minutes, before loading the samples. A 0.8 % agarose gel was cast using agarose (Wealtec) and the GES-system with casting tray, comb, dams-claws and electrophoresis tank (Wealtec). Gels were allowed to set for at least one hour before loading samples. Two different DNA-ladders (500 bp DNA-ladder; Bangalore Genei, Bangladore, India and 1 kb DNA-marker; New England Biolabs), were loaded at each sides of the single and double digested DNA. The DNA was resolved on the gel at 120 V for approximately 70 minutes. Thereafter the gel was soaked in ethidium bromide solution (5 µg/ml; BioRad), for 30 minutes. After a brief soak in ddH_20, the gel was visualised using Dolphin-Doc Plus Image system (Wealtec).

RESULT

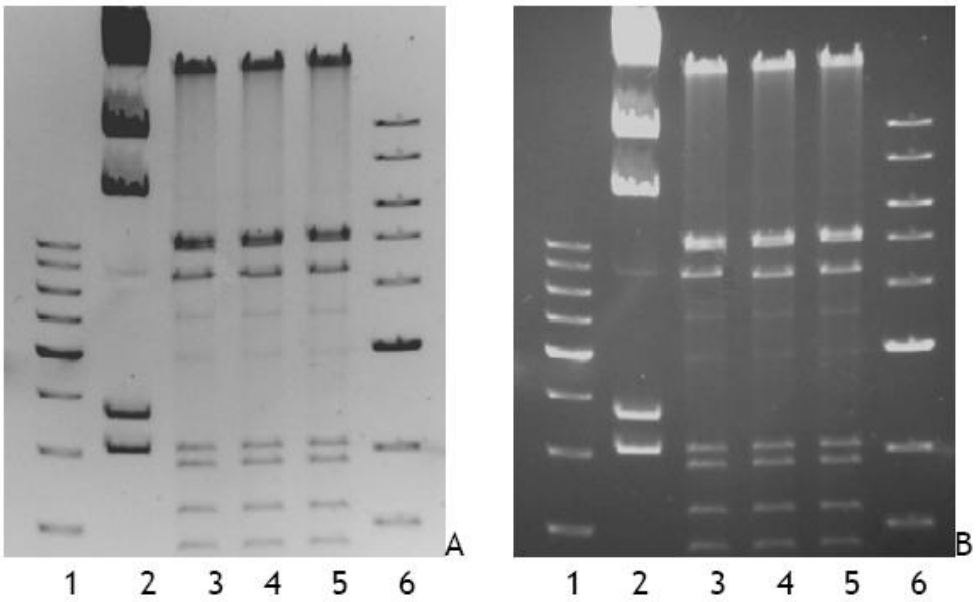

Figure 2 A and B: *Normal (A) and inverted (B) images of restriction digests from single and double digested lambda-DNA, run on a 0.8 % agarose gel. Lane 1: 500 bp DNA-ladder (0.2 µg), lane 2: lambda- DNA cut by HindIII (0.5 µg), Lane 3, 4 and 5: lambda-DNA cut by HindIII and EcoRI(all 0.5µg), Lane 6: 1 kb DNA-ladder(0.2 µg).*

DISCUSSION

Figure 2 shows the cutting pattern of single digested lambda-DNA cut by *Hind*III in lane 2, and double digested DNA cut by *Hind*III and *Eco*RI in lanes 3, 4 and 5.
Larger DNA-fragements have been cut into smaller, appearing as new bands further down in the gel. The digestion appears to have been working under current circumstances; however, there may be several reasons why the reaction sometimes fails. Some restriction enzymes will alter recognition specificity and/or efficiency when operating under sub-optimal conditions, a phenomenon called "star"-activity (Mani *et al.*, 2005b). The reasons for star-activity can be e.g. a high content of glycerol, low salt strength, too high pH or enzyme concentration, absence of Mg^{2+} or traces of organic solvents, such as phenol. As an example, *Eco*RI, which under optimal circumstances cleaves at G|AATTC, will instead cleave at N|AATTN under high pH and low ionic strength, where N represents any nucleotide. Another factor that might impair restriction cutting even though the conditions are correct is *methylation* of DNA. If the target sequence of DNA is methylated, the restriction enzyme will not recognise the site. Methylation is in fact the way bacteria protect their own DNA from being cut by their own restriction enzyme. Bacteria will produce not only a restriction enzyme, but also a specific methyl transferase, methylating those sequences in the bacterial DNA that would be potential targets of the restriction enzyme.
With a temperature range from 0 to 70°C, Wealtec's CB-1 Block Cooler allows for multiple incubation applications in molecular biology. With excellent temperature control, CB-1 is especially suited for enzymatic reactions such as restriction digests. With cooling temperatures closer to zero, CB-1 is also perfect for thawing samples and keeping sensitive enzymes cool.

REFERENCE

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